MONITORING ESSENTIAL OIL QUALITY OF *ETLINGERA SP1* BY GC AND GC-MS

SIVANESAN SHANMUGAM

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Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

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I declare that this thesis entitled “Monitoring Essential Oil Quality of *Etlingera sp1* BY GC and GC-MS” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : …….....

Name of Candidate : …….....

Date : …….....

DEDICATION

Specially dedicated to my late grandmother, family and friends for their love and care…

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Completion and submission of a thesis of this kind involves lots of hard work and sacrifices. This thesis is a result of almost a year of study whereby I have been accompanied and fostered by many. It is a pleasant moment that I have now the opportunity to express my gratitude to all of them.

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ABSTRACT

Etlingera sp.1 of the family *Zingiberaceae* is being studied for changes in its essential oil quality during storage. *Zingiberaceae* comprises about 1200 species of which 1000 is distributed throughout Tropical Asia. Essential oil exists in all species in the genus *Etlingera.* The highest content is usually in the rhizomes of this plant. Essential oil extracted from the rhizomes is primarily used in fragrance making and have high commercial value due to its antibacterial and therapeutic properties. Suitable extraction method needs to be chosen as essential oils are composed of heat-sensitive chemical constituents. In this study, the Clevenger-type hydrodistillation method was employed due to ease of use and milder extracting condition. Essential oil composition was analyzed using GC and GC-MS. Generally, gas chromatographic techniques are used to separate mixtures of chemical constituents into individual components Compounds present were identified using GC-MS while GC was used for weekly analysis. n-Hexane was chosen as solvent as previous studies using n-hexane reportedly produced optimum results. Comparative analysis conducted over a three weeks period indicated that the quality of essential oil exposed to light and temperature underwent minor changes.

ABSTRAK

Etlingera sp.1 dari famili *Zingiberaceae* telah dikaji perubahan ke atas kualiti minyak patinya semasa penyimpanan. *Zingiberaceae* terdiri daripada 1200 spesis dengan seribu darinya tertabur di keseluruhan Asia Tropika. Minyak pati wujud di semua spesis dalam genus *Etlingera.* Kandungan tertinggi minyak pati ialah di dalam rizom tumbuhan. Minyak pati yang diekstrak daripada rizom digunakan di dalam persediaan fragran dan mempunyai nilai kormesial tinggi sebagai antibakteria dan terapeutik. Kaedah pengekstrakan yang sesuai adalah perlu disebabkan komposisi minyak pati adalah sensitif haba. Di dalam kajian ini, metod penyulingan hidro jenis Clevenger digunakan kerana mudah dan kondisi pengekstrakannya yang sederhana. Komposisi minyak pati dianalisa menggunakan GC dan GC-MS. Amnya, teknik kromatografi gas diguna untuk memisahkan campuran kimia kepada kompaun individu. GC-MS diguna untuk mengenalpasti kompaun yang hadir dalam sampel manakala GC diguna untuk analisis mingguan. n-Heksana dipilih sebagai pelarut memandangkan kajian-kajian sebelumnya melaporkan hasil yang optimum dengan penggunaannya. Perbandingan analisis yang dibuat selama tiga minggu mendapati kualiti minyak pati telah mengalami perubahan kecil-kecilan.

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LIST OF ABBREVATIONS AND SYMBOLS

T	=	Temperature
t	=	Time
cm	=	Centimeter
m	=	Meter
g	=	Gram
°C	=	Degree Celcius
K	=	Kelvin
%	=	Percentage
g/mL	=	gram per milliliter
mg/L	=	Milligram per litre
rpm	=	rotation per minute

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CHAPTER 1

INTRODUCTION

1.1 Background

Zingiberaceae, or the family of ginger in layman term, is a family of flowering plants consisting of aromatic lasting herbs with rhizomes, comprising 52 genera and more than 1300 species, distributed throughout tropical Africa, Asia and the Americas (Wikipedia, 2007).The most popular species from this family is the edible ginger of commerce known in Malay as "halia" (scientific name: *Zingiber officinale*).Noticeably, from its total population, around 1000 species occur in tropical Asia.Many species are important ornamental plants, spices, or medicinal plants. Attractive genera include the shell gingers (*Alpinia*), Siam or summer tulip (*Curcuma alismatifolia*), *Globba*, ginger lily (*Hedychium*), *Kaempferia*, torch-ginger *Nicolaia*, *Renealmia*, and ginger (*Zingiber*). Spices include ginger (*Zingiber*), galangal or Thai ginger (*Alpinia galanga* and others), melegueta pepper (*Aframomum melegueta*), myoga (*Zingiber mioga*), turmeric (*Curcuma*), cardamom (*Amomum*, *Elettaria*).

1.2 Problem Statement

Quality of essential oils degrades easily upon exposure to light, air and changing climate. The study is to monitor changes in the essential oil of an *Etlingera* species using

GC-FID and GC-MS under different conditions, namely, temperature and light, over time.

1.3 Objectives

The objective of this study is to perform a comparison study between samples of essential oil obtained from *Etlingera sp.1*.The aim of this experiment is to monitor the changes in essential oil quality *Etlingera sp.1* using GC-FID and GC-MS upon certain length of time to heat and light and to identify the compounds present.

1.4 Research Scope

Variation in *Etlingera sp.1* essential oil sample is being studied for its property changes over 3 weeks period.

CHAPTER 2

LITERATURE REVIEW

2.1 The Genus *Etlingera* - An overview

Kingdom	*Plantae*
Subkingdom	*Viridaeplantae*
Phylum	*Tracheophyta*
Subphylum	*Spermatophytina*
Intraphylum	*Angiosperma*
Division	*Magnoliphyta*
Class	*Liliopsida*
Order	*Zingiberales*
Family	*Zingiberaceceae*
Genus	*Etlingera*
Species	*sp.1*
Scientific name	*?*
Common name	?

Figure 2.1: Taxonomy of Ginger (Farlex, 2004)

The genus *Etlingera* is distributed from India to the Pacific Islands with Borneo and New Guinea seem to be the most popular location of this species. To date, botanists estimate about 10 species of *Etlingera* distributed throughout Peninsular Malaysia and Singapore. The varying shades of pink and red colours of bracts and flowers make *Etlingera* species very attractive plants. Plants of *Etlingera* have various traditional and commercial uses. While comprehensive morphological examination is being carried out by the botanist at Universiti Malaya, the specimen that used is this study will be named *Etlingera* sp. 1

Even though we do not have specific information on this species, we can expect similarities from previous researches done on the other species which belong to the same genus, *Etlingera.* For example, In Sabah, Malaysia, the hearts of young shoots, flower buds, and fruits of *E. elatior, E. rubrolutea,* and *E. littoralis* are consumed by indigenous communities as condiment, eaten raw or cooked (Noweg, Abdullah, & Nidang, 2003). In Thailand, fruits and cores of young stems of E. littoralis are edible, and flowers of E. maingayi are eaten as vegetables (Sirirugsa, 1999). Inflorescences of *E. elatior* are widely cultivated throughout the tropics as spices for food flavoring and as ornamentals. They are commonly used as ingredients of dishes such as laksa asam, nasi kerabu, and nasi ulam in Peninsular Malaysia (Larsen, Ibrahim, Khaw, & Saw 1999). Farms in Australia and Costa Rica are cultivating the species and selling its inflorescences as cut flowers (Larsen et al., 1999). In Malaysia, fruits of *E. elatior* are used traditionally to treat earache, while leaves are applied for cleaning wounds (Ibrahim & Setyowati, 1999). Leaves of *E.* elatior, mixed with other aromatic herbs in water, are used by post-partum (depression after pregnancy) women for bathing to remove body odor. Phytochemical studies on rhizomes of E. *elatior* led to the isolation of two new and six known compounds of diarylheptanoids, labdane diterpenoids, and steroids (Habsah et al., 2005). Ethanolic extracts from the flower shoots of *E. elatior* have antimicrobial activity and are cytotoxic to heal cells (Mackeen et al., 1997).Past studies on the antioxidant activity of ginger species were confined to rhizomes (Jitoe et al., 1992; Habsah et al., 2000; Zaeoung, Plubrukarn, & Keawpradub, 2005). Their rhizomes have been reported to contain antioxidants. (Chan et al., 2007)

2.1.1 Ecology

Species of *Etlingera* can be more than 5 m tall and become dominant in gaps (*E. megalocheilos, E. brevilabrum, E. coccinea, E. fimbriobracteata*).The reproductive

biology has been investigated by Ms. Louise Pedersen (Univ. Copenhagen) who found that spider hunters are important pollinators (Axel, 2007).

2.1.2 Medicine

The species sp.1 that is being studied is likely to have medicinal and therapeutic properties just like other members of the *Zingiberaceae* family. Many previous studies have proved this to be true. An organic extract of *Etlingera* aff. rosea B.L. Burtt & R.M. Sm. (*Zingiberaceae*) was found to exhibit significant cytotoxic activity when evaluated against a panel of human cancer cell lines. Leaves of *Etlingera* species inhibited Gram-positive bacteria. With promising antioxidant and antibacterial properties, leaves of *Etlingera* species have great potential to be developed into natural preservatives and herbal products, applicable to the food and pharmaceutical industries. Unlike the commercial use of rhizomes, the harvesting of leaves does not result in destructive sampling of plants (Chan et al, 2007).

2.1.3 Selected plants from the genera *Etlingera*

Figure 2.2: *Etlingera Elatior* “Yamamoto” (http://www.alohatropicals.com)

Figure 2.3: *Etlingera Elatior* "Thai Queen" (http://www.alohatropicals.com)

Figure 2.4: *Etlingera hemisphaerica* "Helani Tulip" (http://www.alohatropicals.com)

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2.1.4 Essential oil

The essential oil of a material is the name given to the mixtures of substances extracted from a biological system and contains the essential components that provide the characteristic smell or flavor of that material. They are also known as volatile or refined oils or simply as the oil of the plant material from which they were extracted. The term essential indicates that the oil carries distinctive fragrance (essence) of the plant, importantly or core substance.For example, peppermint oil, patchouli oil, jasmine oil and etc. Essential oils are usually a highly complex mixture of a wide variety of substances.

The oil may often be simulated by a fairly simple mixture of artificial compounds blended in the same percentage as the original oil but the aroma or taste often lacks the validity of the original essential oil (Yoshiro, 1976). Essential oils are extracted by different methods. The material is sometimes leached with water and the oil steam distilled from the aqueous mixture. The natural material may also be solvent extracted and the oil recovered by distillation. Extraction must be done with care as many of the components of essential oils are temperature sensitive. Essential oils analysis without the use of gas chromatography would be extremely difficult. Prior to the technique being developed, only the major components of the oils could be separated, achieved by distillation with high efficiency columns (Holttum, 1965).

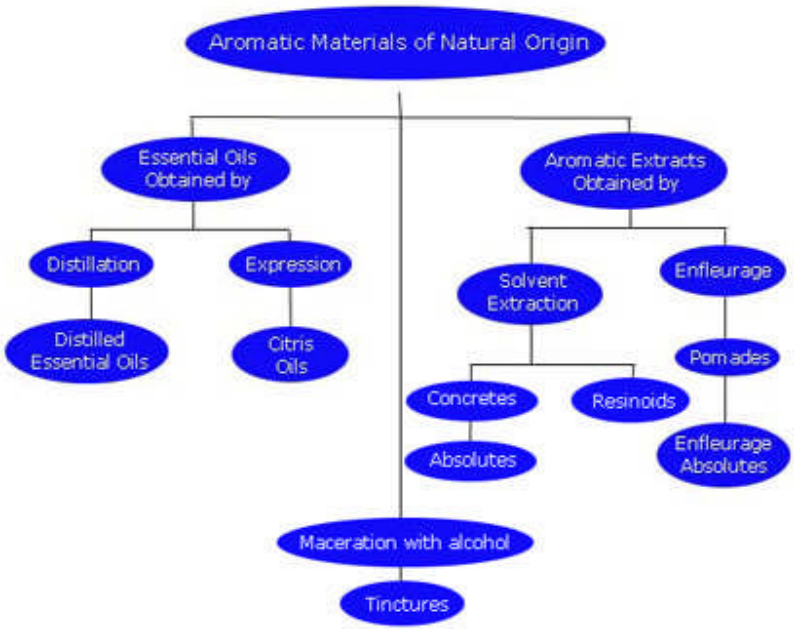

Figure 2.13: Essential Oil extraction chart (www.healing.about.com)

The extraction method depends on the plant material as well as the type of end product that is desired. Essential oils been used medicinally at different periods in the past. Medical applications proposed by those who sell medicinal oils vary from skin treatments to remedies for cancer, and are often based on historical use of these oils for these purposes.

Interest in essential oils has revived in recent times, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects.

Berries	Leaves	Flowers
• Allspice • Juniper **Seeds** • Almond • Anise • Nutmeg oil **Wood** • Camphor • Cedar • Rosewood • Sandalwood **Rhizome** • Ginger	• Basil • Bay leaf • Cinnamon • Common sage • Eucalyptus • Lemon grass • Oregano • Patchouli • Peppermint • Pine • Rosemary • Spearmint **Resin** • Frankincense • Myrrh	• Chamomile • Clary sage • Clove • Geranium • Hyssop • Jasmine • Lavender • Marjoram • Orange • Rose • Ylang-ylang **Peel** • Bergamot • Lime • Orange • Tangerine **Root** • Valerian

Figure 2.14: Plant bases of various Essential Oils (www.healing.about.com)